Biomaterials 32 (2011) 3611–3619

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

High-throughput screening of microscale pitted substrate topographies for enhanced nonviral transfection efficiency in primary human fibroblasts

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ARTICLE INFO

Article history:
Received 30 December 2010
Accepted 13 January 2011
Available online 21 February 2011

Keywords:
Surface topography
Gene transfer
Combinatorial screening
Microstructure
Cell spreading
Fibroblast

ABSTRACT

Optimization of nonviral gene delivery typically focuses on the design of particulate carriers that are endowed with desirable membrane targeting, internalization, and endosomal escape properties. Topographical control of cell transfectability, however, remains a largely unexplored parameter. Emerging literature has highlighted the influence of cell–topography interactions on modulation of many cell phenotypes, including protein expression and cytoskeletal behaviors implicated in endocytosis. Using high-throughput screening of primary human dermal fibroblasts cultured on a combinatorial library of microscale topographies, we have demonstrated an improvement in nonviral transfection efficiency for cells cultured on dense micropit patterns compared to smooth substrates, as verified with flow cytometry. A 25% increase in GFP^+ cells was observed independent of proliferation rate, accompanied by SEM and confocal microscopy characterization to help explain the phenomenon qualitatively. This finding encourages researchers to investigate substrate topography as a new design consideration for the optimization of nonviral transfection systems.

1. Introduction

The promise of gene medicine is intimately linked to the efficiency of nonviral transfection. Mechanistic understanding of the nonviral gene delivery process remains incomplete, and so is the structure-function relationship of nonviral vectors. Currently, particulate parameters of DNA nanocomplexes cannot fully account for the differences in nonviral transfection efficiency observed between various gene delivery systems (reviewed in [1]). At the same time, substrate parameters including stiffness [2,3], co-presentation of adsorbed vectors with ECM and serum proteins [4–6], and surface chemistry [7,8] have been demonstrated to strongly influence the uptake and expression of nonviral vectors, though comparatively little focus has been placed on their design. These differences are often attributed to vector-substrate interactions - using substrates as depots for the capture and/or controlled release of nonviral gene carriers, thereby increasing their local concentration at cell surfaces [9–11]. However, increasing importance is being placed on biochemical and physical cell-substrate interactions, which may prime cells to become more readily transfected [3,12,13]. For example, collagen-coated surfaces inhibit transfection of mesenchymal stem cells (MSCs) following bolus delivery of polyplexes compared to those cultured on uncoated surfaces, whereas fibronectin coating increases transgene expression by enhancing clathrin-mediated endocytosis [12]. Physically, stiffer substrates support more efficient transfection, attributed to enhanced proliferation on hard surfaces [3], which is believed to increase nuclear accessibility to nonviral vectors during cell division [14].

Substrate topography may also be playing a role in the transfection process, through cell-substrate and/or vector-substrate interactions. Patterned topography has been observed to engender a number of phenotypic changes in cells, many of which with implications in the delivery of nonviral vectors that have yet to be investigated. Cells interact with their substrates through integrins, a family of transmembrane receptors that bind to an assortment of extracellular matrix proteins. Integrin binding and clustering initiates the assembly of focal adhesion complexes [15]. Substrate topography and chemistry alter the amount and conformation of integrin ligands [16–20], and may form the general basis for many if not all subsequent topographical effects on cell behavior [19–21].

Altered cell spreading is one of the most often-observed effects of culture on nano- and microtopographic surfaces. The heterogeneous presentation of extracellular matrix proteins adsorbed to topography supports varying degrees of integrin engagement, leading to varying degrees of cell spreading [15–17] nuclear

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doi:10.1016/j.biomaterials.2011.01.040

deformation [21–24], and subsequent changes in genomic expression profiles [24,25]. The act of integrin engagement and spreading itself may have an effect on the clathrin- and caveolae-mediated uptake of nonviral vectors, two of the primary routes along which particulate gene carriers are delivered to the nucleus [26–29]. This rationale is supported by studies describing molecular links between cell substrates, focal adhesions, and the cytoskeletal and endocytic machineries [30–35].

Observing the influence of topographical cues on many cell phenotypes including proliferation [36–38], spreading, cytoskeletal organization, and endocytosis, we hypothesize that substrates with micropatterned topographies may also influence the transfectability of interacting cells. In this study, we investigated the transfection of normal human dermal fibroblasts (NHDFs) by GFP-encoding lipoplexes on a topographical library comprising 160 patterns of square and/or circular pit geometries, systematically varied with respect to size, spacing, and arrangement in the 1–6 μm range. Morphology and proliferation were also documented to further examine the observed differences in transfection efficiency. Patterns with elevated transfection levels on the library (3×3 mm^2 each pattern) were replicated as large (2×2 cm^2) single-pattern substrates for analysis by flow cytometry, luciferase assay, and characterization with SEM and confocal microscopy.

2. Materials and methods

2.1. Preparation of 13 × 13 pattern topographical library

Topographical libraries (arrays) were prepared as described in [39], using a standard lithography process. Briefly, a resist without hard bake was etched with Cl_2, HBr and NF_3 to produce sidewall angles of 85° with etch rate non-uniformity of 2–3% (max–min). After removal of the resist and cleaning, the surface was sputter-coated with 100 nm of tantalum at a rate of 50 nm min^{-1}, producing a 13×13 master library of post topographies. To create substrates for cell culture, Sylgard 184 polydimethylsiloxane (PDMS) (Dow Corning, Midland, MI) was mixed at a curing agent/base ratio of 1.05/10 w/w, degassed in a vacuum chamber, cast onto the metal master, and cured overnight at 47 °C. PDMS arrays were peeled from the metal master and used for cell culture. These arrays were composed of 10 distinct pit morphologies (A–J) with: pit size (X) and edge-to-edge spacing (Y) iterated combinatorially through values of 1, 2, 4, and 6 μm (Fig. 1), a uniform depth of 2.4 μm, and with 3×3 mm^2 patterned area for each. Pattern K did not support robust NHDF adhesion, and was excluded from all analyses. Smooth control regions were present in the middle of the array, and in the boundaries between patterns. Large area (2×2 cm^2) metal masters of the single patterns F(X,Y) = F(1,4) and F(4,1) were also produced following the same lithographical process to facilitate analysis by flow cytometry and luciferase assay. Pitted PDMS replicates of these "single-pattern" substrates, as well as smooth PDMS substrates produced from smooth silicon wafers, were stamped into 2 cm^2 circular disks, affixed to the bottom of 24-well tissue culture plates (Falcon, Heidelberg, Germany) with 50 μL PDMS, and cured overnight.

2.2. Cell culture

Adult normal human dermal fibroblasts (NHDFs) (Lonza, Basel, Switzerland) were cultured in high glucose Dulbecco's Modified Eagle's Medium (GIBCO 11960-044) (Invitrogen, Carlsbad, CA) supplemented with 20% Premium Select FBS (Atlanta Biologicals, Lawrenceville, GA), 25 μg mL^{-1} gentamicin (Invitrogen), and $1 \times$ GlutaMAX, non-essential amino acids, sodium pyruvate, and β-mercaptoethanol (Invitrogen), at 37 °C and 5% CO_2. NHDFs were passaged a maximum of six times prior to experimentation. PDMS substrates were washed with 70% ethanol and air-dried twice, then washed with sterile deionized (DI) water and air-dried twice again. 25 μg mL^{-1} human plasma fibronectin (BD, Franklin Lakes, NJ) in DI water was adsorbed to PDMS substrates for 1 h at room temperature (RT) before seeding NHDFs at a density of 7500 cells cm^{-2} for quantitative analyses, and 3750 cells cm^{-2} for visualization by SEM and confocal microscopy.

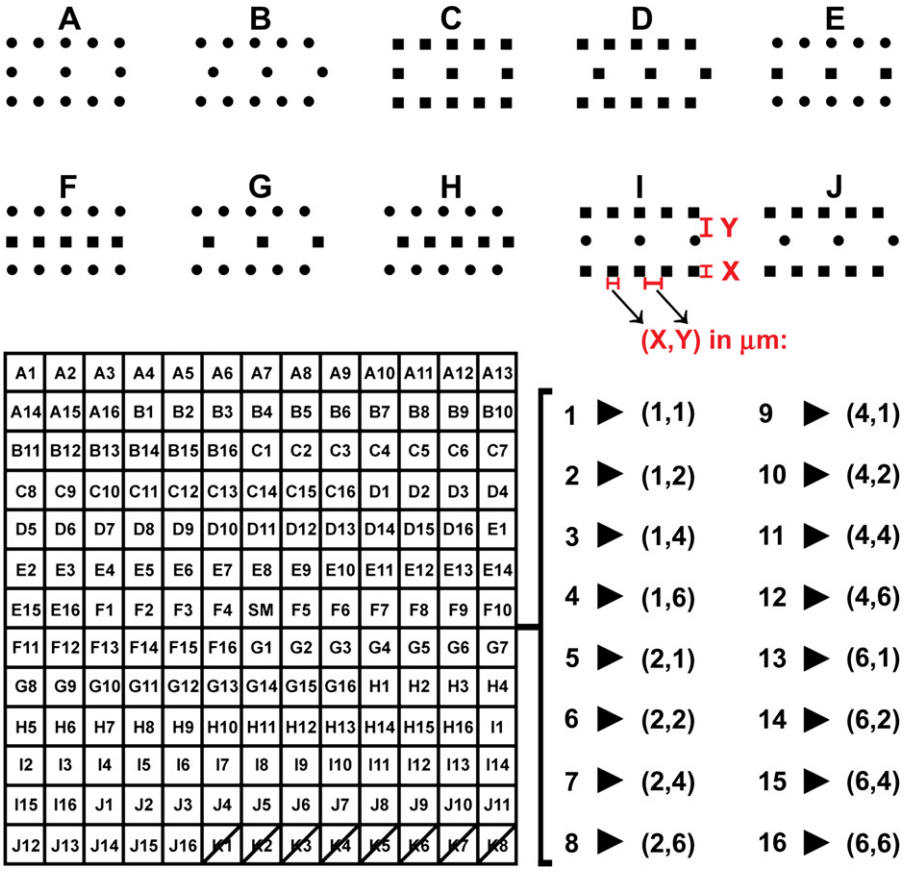

Fig. 1. Topographical library details: 10 pit morphologies (A–J) were replicated with 16 different combinations of size (X, 1–6 μm) and spacing (Y, 1–6 μm), giving 160 unique patterned PDMS substrates for cell growth, each with a uniform pit depth of 2.4 μm. Smooth regions were present in the center of the array (SM), and in the regions between patterns. Pattern K was excluded from all analyses due to poor cell attachment.